

1 What is claimed is:
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4 1. A recombinant immunotoxin polypeptide and pharmaceutically
5 acceptable salts thereof comprising a CD3-binding domain and a
6 *Pseudomonas* exotoxin (PE) mutant, said PE mutant having ADP-
7 ribosylating and translocation functions but substantially
8 diminished cell-binding ability.
9

10 2. A recombinant immunotoxin polypeptide and pharmaceutically
11 acceptable salts thereof according to claim 1 wherein the CD3-
12 binding domain comprises an anti-CD3 antibody or CD3-binding
13 fragment thereof.
14

15 3. A recombinant immunotoxin polypeptide polypeptide and
16 pharmaceutically acceptable salts thereof according to claim 2
17 wherein the anti-CD3 antibody or CD3-binding fragment thereof
18 binds an epitope on the ϵ chain of human CD3.
19

20 4. A recombinant immunotoxin polypeptide and pharmaceutically
21 acceptable salts thereof according to claim 2 wherein the anti-
22 CD3 antibody or CD3-binding fragment thereof binds an epitope
23 formed by the ϵ and γ chains of human CD3.
24

25 5. A recombinant immunotoxin polypeptide and pharmaceutically
26 acceptable salts thereof according to claim 2 wherein the CD3-
27 binding domain comprises a Fab fragment of an anti-CD3 antibody.
28

29 6. A recombinant immunotoxin polypeptide and pharmaceutically
30 acceptable salts thereof according to claim 2 wherein the CD3-
31 binding domain comprises the Fv region, or a CD3-binding
32 fragment thereof, of an anti-CD3 antibody.
33

1 7. A recombinant immunotoxin polypeptide and pharmaceutically
2 acceptable salts thereof according to claim 2 wherein the CD3-
3 binding domain comprises monoclonal antibody UCHT-1 or a CD3-
4 binding fragment thereof.

5
6 8. A recombinant immunotoxin polypeptide polypeptide and
7 pharmaceutically acceptable salts thereof according to claim 2
8 wherein the CD3-binding domain comprises (the Fv region, or a
9 CD3-binding fragment thereof, of an antibody selected from:
10 monoclonal antibody UCHT-1, an antibody having a variable region
11 which is at least 80% identical to the variable region of UCHT-
12 1, an antibody having complementarity-determining regions
13 identical with those of UCHT-1 and having at least one sequence
14 segment of at least five amino acids of human origin, and an
15 antibody competing with UCHT-1 for binding to human CD3 antigen
16 at least about 80% as effectively on a molar basis, and having
17 at least one sequence segment of at least five amino acids of
18 human origin.

19
20 9. A recombinant immunotoxin polypeptide and pharmaceutically
21 acceptable salts thereof according to claim 2 wherein the CD3-
22 binding domain comprises a single chain Fv of an anti-CD3
23 antibody.

24
25 10. A recombinant immunotoxin polypeptide and pharmaceutically
26 acceptable salts thereof according to claim 8 wherein (the Fv
27 region) is a single chain Fv.

28
29 11. A recombinant immunotoxin polypeptide and pharmaceutically
30 acceptable salts thereof according to claim 10 wherein the CD3-
31 binding domain comprises a single chain Fv of UCHT-1.

1
2 12. A recombinant immunotoxin polypeptide and pharmaceutically
3 acceptable salts thereof according to claim 1 comprising a
4 single chain Fv of UCHT-1 fused to a PE mutant essentially
5 deleted of its cell-binding domain.

6
7 13. A recombinant immunotoxin polypeptide and pharmaceutically
8 acceptable salts thereof according to claim 12 wherein the PE
9 mutant is PE38.

10
11 14. A recombinant immunotoxin polypeptide and pharmaceutically
12 acceptable salts thereof according to claim 1 consisting
13 essentially of the single chain Fv of an anti-human CD3 antibody
14 fused via the carboxy terminus thereof to a PE mutant
15 essentially deleted of its cell-binding domain.

16
17 15. A recombinant immunotoxin polypeptide and pharmaceutically
18 acceptable salts thereof according to claim 14 having the
19 formula $V_L - L - V_H - C - PE$ mutant.

20
21 16. A recombinant immunotoxin polypeptide and pharmaceutically
22 acceptable salts thereof according to claim 15 wherein V_L and V_H
23 are derived from UCHT-1 and the PE mutant is PE38.

24
25 17. A recombinant immunotoxin polypeptide selected from
26 polypeptides having residues 1-601, 2-601 and 3-601 of Sequence
27 ID. NO: 1, homologs of said polypeptides which are at least 80%
28 identical thereto and their pharmaceutically acceptable salts.

29
30 18. A recombinant immunotoxin polypeptide according to claim 17
31 having residues 3-601 of SEQ. ID No:1 and its pharmaceutically
32 acceptable salts.

33

1 19. A nucleic acid molecule encoding the recombinant
2 immunotoxin polypeptide of claim 1.

3
4 20. A method of preparing a recombinant immunotoxin polypeptide
5 of claim 1.

6
7 21. A method for treatment or prophylaxis of T-cell mediated
8 disorders in a patient comprising administering to a patient in
9 need thereof a therapeutically effective amount of a recombinant
10 immunotoxin polypeptide or its pharmaceutically acceptable salt
11 according to claim 1.

12
13 22. A method for treatment or prophylaxis of organ
14 transplantation rejection in a transplant patient comprising
15 administering to the patient a therapeutically effective amount
16 of a recombinant immunotoxin polypeptide or its pharmaceutically
17 acceptable salt according to claim 1.

18
19 23. A method for treatment or prophylaxis of autoimmune disease
20 in a patient comprising administering to the patient a
21 therapeutically effective amount of a recombinant immunotoxin
22 polypeptide or its pharmaceutically acceptable salt according to
23 claim 1.

24
25 24. An autologous therapy for treating or preventing a T-cell
26 mediated disorder or condition in a patient, comprising:

27 (a) recruiting from the patient a cell population
28 comprising CD3-bearing cells;

29 (b) treating the cell population with a recombinant
30 immunotoxin polypeptide or its pharmaceutically acceptable salt
31 according to claim 1 to at least partially deplete said cell
32 population of CD3-bearing cells; and

33

1 (c) reinfusing the treated cell population into the
2 patient.

3
4 25. A method for treatment or prophylaxis against graft versus
5 host disease in patient to undergo a bone marrow transplant
6 comprising:

7 (a) providing an inoculum comprising isolated bone marrow
8 and/or stem cell-enriched peripheral blood cells of a suitable
9 donor treated with a T-cell depleting effective amount of a
10 recombinant immunotoxin polypeptide or its pharmaceutically
11 acceptable salt according to claim 1; and

12 (b) transplanting the inoculum into the patient.

13
14 26. A method for the treatment or prophylaxis or treatment of
15 transplant rejection in a patient to undergo a bone marrow
16 transplant comprising:

17 (a) reducing the levels of viable CD3-bearing cell
18 population in the patient;

19 (b) providing an inoculum comprising isolated bone marrow
20 and/or stem cell-enriched peripheral blood cells of a suitable
21 donor treated with a T-cell depleting effective amount of a
22 recombinant immunotoxin polypeptide or its pharmaceutically
23 acceptable salt according to claim 1; and

24 (c) introducing the inoculum into the patient, and
25 thereafter optionally administering a recombinant immunotoxin
26 polypeptide according to claim 1 to the patient to further
27 deplete donor and patient T cells.

28
29 27. A method of conditioning a patient to be transplanted with
30 cells, or a tissue or organ of a donor, the method comprising:

31 (a) depleting the CD3-bearing cell population in the
32 patient;

1 (b) providing an inoculum comprising isolated bone marrow
2 and/or stem-cell enriched peripheral blood cells of the donor
3 treated with a T-cell depleting effective amount of a
4 recombinant immunotoxin polypeptide or its pharmaceutically
5 acceptable salt according to claim 1;

6 (c) introducing the inoculum into the patient; and

7 (d) transplanting the donor cells, tissue or organ into the
8 patient.

9
10 28. A method according to claim 21 comprising co-administering
11 the recombinant immunotoxin polypeptide or its pharmaceutically
12 acceptable salt with at least one other pharmaceutical agent
13 selected from cyclosporin A, rapamycin, 40-O-(2-hydroxy)ethyl
14 rapamycin (RAD), FK-506, mycophenolic acid, mycophenolate mofetil
15 (MMF), cyclophosphamide, azathioprene, leflunomide, mizoribine, a
16 deoxyspergualine compound or derivative or analog, 2-amino-2-[2-
17 (4-octylphenyl)ethyl]propane-1,3-diol, corticosteroids, anti-LFA-
18 1 and anti-ICAM antibodies, and other antibodies that prevent co-
19 stimulation of T cells.

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21 29. A pharmaceutical composition comprising a recombinant
22 immunotoxin polypeptide or its pharmaceutically acceptable salt
23 according to claim 1 in a pharmaceutically acceptable carrier.

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